Microbiological Characteristics of Beef Tongues and Livers as Affected by Temperature-Abuse and Packaging Systems

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ABSTRACT

Effects of various handling, packaging, temperature-abuse and storage conditions were determined on the microbiological characteristics of beef livers and tongues. These organs were evaluated: (a) initially following slaughter, (b) immediately following the frozen storage period of 2-4 weeks at -29°C and (c) following a simulated shipping-temperature abuse of 24 h at 22-28°C followed by 13 days of storage at -1 ± 0.5°C. Initial counts (log/cm\textsuperscript{2}) of coliforms, coagulase-positive \textit{Staphylococcus aureus} and \textit{Clostridium perfringens} ranged from 0.19-1.37. Generally, neither freezing nor temperature-abuse had a significant effect on these microorganisms. Vacuum-packaged beef tongues and livers, generally, had lower bacterial counts than did either naked or polyvinyl chloride film-wrapped products. Generally, it was observed that abusive storage conditions were determined on the microbiological spoilage problems when compared with vacuum packaging.

The United States currently exports approximately 190,000 tons of variety meats (worth 110 million dollars) annually to Europe. This market is a valuable outlet for U.S. variety meats since their consumption is very limited in the United States. However, there are several problems accompanying the export of U.S. variety meats to Europe; these include: (a) failure to adhere to product quality standards and specifications, (b) inappropriate processing, chilling and freezing procedures, (c) uncontrolled systems of product assembly for export by brokers and (d) deterioration of product and packaging during transit.

Only limited information on microbial and organoleptic spoilage patterns of variety meats has appeared in the literature. Microbiological contamination on porcine livers is predominantly on the surfaces and freezing does not change the spoilage characteristics (6). Beef livers were found organoleptically unacceptable after 7-10 days of storage at 5°C (8,15). This was probably due to a souring-type spoilage and bacterial levels of 7.2-7.8 × 10\textsuperscript{7} organisms/g. Variety meats produced with a minimum of handling (12), and thus having a relatively low initial number of microbes (10\textsuperscript{4} organisms/cm\textsuperscript{2}), can be stored 1-2 weeks longer under vacuum packaged conditions than when microbe levels were higher (10\textsuperscript{5}-10\textsuperscript{6} organisms/cm\textsuperscript{2}).

Additional research is necessary to determine the effects that standard processing, packaging and transit procedures have on the microbiological and shelflife characteristics of variety meats. The purpose of this study was to determine quantitative and qualitative changes in microorganisms on variety meats as a result of various packaging systems and simulated temperature-abuse conditions.

MATERIALS AND METHODS

Sample acquisition

Beef tongues and livers from freshly slaughtered and dressed cattle were selected and purchased at two slaughtering plants. At plants I and II (Trench and Sons, Elk City, MD), sampling occurred both in mid-summer (Plant I) and mid-winter (Plant II). At Plant III (Shen Valley Meat Packers, Timberville, VA), sampling occurred only in mid-winter. At the plants, the variety meats were individually wrapped in plastic bags and placed in cardboard boxes immediately following evisceration and washing. Samples were brought back to the Meat Science Research Laboratory, USDA, Beltsville, MD. Twenty seven of the 75 beef liver samples and 25 of the 73 beef tongue samples from both plants were analyzed by surface swabbing immediately upon arrival to determine initial microbiological numbers. Samples were 6 to 8 h post-evisceration upon arrival at Beltsville, MD. Between slaughter and sampling, the samples were held between 6 and 17°C. Following sampling, all of the variety meat samples were placed in a cooler at 3°C and held overnight. The following day, 36 of the samples which were not initially sampled were divided into the various abuse treatment-packaging systems. The remaining samples were frozen and served as non-temperature-abused controls at the conclusion of the temperature-abuse.

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Three separate packaging systems were used: (a) vacuum packaging in Cryovac B 620, polyvinylidene bags (W. R. Grace and Co., Duncan, SC), with evacuation in a Multivac, (model #3696-24, type A 6500) vacuum packager (b) wrapping in a single layer of polyvinyl chloride (PVC) film (Alcoa), so as to exclude as much air as possible or (c) a naked (no wrapping material) product. The packaging materials had the following permeability characteristics, polyvinylidene chloride bag: Oxygen Transmission Rate (OTR) = 35 cc/m²/24 h/ atm. Moisture Transmission Rate (MVTR) = 7.6 g/m²/24 h. PVC film: OTR = 15,200 cc/m²/24 h/23°C; MVTR = 380 g/m²/24 h/23°C. All samples were wrapped or packaged individually. Packaged variety meats were then placed into 52 cm x 40 cm x 15 cm boxes with polyethylene liners. Beef tongues were packed six per box and beef livers were packed two per box. The boxes were then placed in a freezer at -29°C ± 2°C where they were held 2-4 weeks before the onset of the simulated shipping-temperature abuse.

Sampling procedure

A sterile, dacron-tipped swab, moistened in 0.1% sterile peptone (Difco) diluent was used to remove bacteria from 12.3 cm² of the surface, according to the procedures of Lazarus et al. (10). Three separate 12.3 cm² areas were swabbed with individual sterile swabs. The three swabs were placed into 10 ml of 0.1% peptone diluent and appropriate serial dilutions were made.

Salmonella detection

The presence of Salmonella was assessed according to the procedures of Poelma and Stilliker (14). Other microbiological procedures are outlined and summarized in Table 1.

Determination of pH values

The pH determinations were made according to the procedures of Cia and Marsh (2). These samples were taken from the product concurrently with the microbial samples. Samples were also removed for analysis following the temperature-abuse and control treatments.

Simulated shipping and temperature-abuse

Boxes containing variety meats were removed from frozen storage and placed on a 122 cm x 102 cm plastic shipping pallet in three layers. Each layer consisted of six boxes. The pallet contained 18 boxes in total. Regions on the pallet not occupied by variety meat sample boxes were substituted with boxes containing 40-lb. ice blocks enclosed in polyethylene bags. The pallet was placed outside of the laboratory in a shaded area for 24 h where ambient temperature ranged from 22-28°C. This procedure simulated conditions variety meats might go through during assembly and loading into the cargo hold of a ship (9). During winter months, pallets were assembled inside a building where the ambient temperature was artificially maintained at 22-28°C. Following this simulated temperature abuse, the pallet was transferred into a cooler for 13 days where the temperature was maintained at ±1°C ± 0.5°C. This procedure simulated the time-span the product would be in the cargo hold of a ship during overseas transport from ports in the Gulf of Mexico in the United States to ports in Europe. At the end of this period, samples were retested for microbiological characteristics. An additional set of samples was removed from frozen storage at the time of the termination of the shipping and temperature-abuse. These samples did not receive the temperature-abuse and were used as controls to determine the effects of the frozen storage, without temperature-abuse, on microbiological numbers. Beef tongues and livers were held 24 h at ±1.0 ± 0.5°C for thawing and then analyzed for microbiological numbers. This 24-h thawing period was necessary for minimal surface thawing required for swabbing of surfaces.

Statistical analysis

Analysis of variance (16) was used to test the effect of temperature-abuse, packaging treatment, variation in plant procedures and all

<table>
<thead>
<tr>
<th>TABLE 1. Microbiological procedures.</th>
<th>Medium</th>
<th>Plating Technique</th>
<th>Incubation</th>
<th>Confirmatory Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Aerobic Plate Count (35 C)</td>
<td>Plate Count Agar</td>
<td>Pour Plate</td>
<td>35 C for 48 hr</td>
<td>...</td>
</tr>
<tr>
<td>2) Aerobic Plate Count (20 C)</td>
<td>Plate Count Agar</td>
<td>Pour Plate</td>
<td>20 C for 5 days</td>
<td>...</td>
</tr>
<tr>
<td>3) Aerobic Plate Count (7 C)</td>
<td>Plate Count Agar</td>
<td>Pour Plate</td>
<td>7 C for 10 days</td>
<td>...</td>
</tr>
<tr>
<td>4) Coliform Count</td>
<td>Violet Red Bile Agar</td>
<td>Pour Plate with overlay</td>
<td>35 C for 24 hr</td>
<td>Growth and gas evolution in Brilliant Green Bile Broth within 48 hr at 35 C</td>
</tr>
<tr>
<td>5) Coagulase-positive Staphylococcus aureus</td>
<td>Three-tube MPN in trypticase soy broth with 10% NaCl</td>
<td>Positive tubes streaked onto Baird-Parker agar</td>
<td>35 C for 48 hr each</td>
<td>Coagulase test</td>
</tr>
<tr>
<td>6) Clostridium perfringens</td>
<td>TSC Agar</td>
<td>Spread Plate with overlay</td>
<td>35 C for 42 hr</td>
<td>Growth and gas evolution in fluid thioglycollate, nonmotility, nitrite and gelatinase test</td>
</tr>
<tr>
<td>7) KF Streptococcal count</td>
<td>KF Streptococcus Agar</td>
<td>Pour Plate</td>
<td>35 C for 48 hr</td>
<td>...</td>
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<tr>
<td>8) Anaerobic Count</td>
<td>Plate Count Agar</td>
<td>Pour Plate</td>
<td>35 C for 48 hr</td>
<td>...</td>
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<tr>
<td>9) Yeast and Mold Count</td>
<td>Plate Count Agar with 100 ppm chlorotetracycline HCL and 100 ppm chloramphenicol</td>
<td>Pour Plate</td>
<td>20 C for 5 days</td>
<td>...</td>
</tr>
<tr>
<td>10) Total Enterobacteriaceae Count</td>
<td>Violet Red Bile Agar with 1% glucose</td>
<td>Pour Plate with overlay</td>
<td>35 C for 24 hr</td>
<td>...</td>
</tr>
</tbody>
</table>
TABLE 2. Microbial counts on beef tongues before and after specified handling and storage conditions.

<table>
<thead>
<tr>
<th>Handling and storage conditions</th>
<th>35 C APC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>20 C APC</th>
<th>7 C APC</th>
<th>Coagulase-positive Staphylococcus aureus</th>
<th>Coliform</th>
<th>Clostridium perfringens</th>
<th>KF Streptococcal</th>
<th>Anaerobic</th>
<th>Yeast &amp; Mold</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INITIAL</strong></td>
<td>4.20&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>3.61&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.32&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.91&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.65&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>1.17&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td><strong>ABUSE&lt;sup&gt;c&lt;/sup&gt;</strong></td>
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<tr>
<td>Vacuum packaging</td>
<td>3.56&lt;sup&gt;h&lt;/sup&gt;</td>
<td>4.08&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.11&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.56&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.34&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Film wrapping, (PVC)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.97&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.94&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.78&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.87&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.22&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Naked (no wrapping material)</td>
<td>5.43&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.82&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.81&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.12&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.46&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.69&lt;sup&gt;f&lt;/sup&gt;</td>
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<td><strong>CONTROL&lt;sup&gt;d&lt;/sup&gt;</strong></td>
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<tr>
<td>Vacuum packaging</td>
<td>3.58&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>3.38&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.99&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.85&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Film wrapping, (PVC)</td>
<td>3.17&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.54&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.59&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Naked (no wrapping material)</td>
<td>3.44&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.59&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.53&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.11&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.15&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Counts = log<sub>10</sub>/cm<sup>2</sup>.
<sup>b</sup>APC = Aerobic plate count.
<sup>c</sup>Two-four weeks storage at -29 C, followed by a simulated shipping-temperature abuse of 24 h at 22-28 C, followed by 13 days storage at -1 ± 0.5 C.
<sup>d</sup>Two-four weeks storage at -29 C.
<sup>e</sup>PVC = Polyvinyl chloride.
<sup>f</sup><sup>gh</sup>Means in the same column with different letters are significantly different at P<0.05.

n = 25 tongues for initial, 12 for abuse and 4 for control conditions.
relevant interactions. When the analysis of variance revealed a significant ($P<0.05$) effect, Duncan's Multiple Range test for mean separation was employed (16).

RESULTS AND DISCUSSION

Beef tongues

Microbial analyses for beef tongues after the simulated shipping and temperature-abuse are presented in Table 2. Highest aerobic plate counts (APC, 35°C) were observed on product either film-wrapped (PVC) or naked (no wrapping material) following abuse. The APC at 35 and 20°C for tongues which were vacuum-packaged and abused, were not significantly different from the initial counts or any of the control treatment counts. The initial APC at 20°C were approximately one log cycle lower than counts previously obtained on beef tongues (13). As with the APC at 35°C, the highest APC at 20°C were found for the naked and film-wrapped product following abuse. Highest APCs at 7°C were observed on naked and film-wrapped product following abuse. They were significantly ($P<0.05$) higher than the surface bacterial counts of the remaining treatments. Counts (7°C APC) on the abused vacuum-packaged product were also significantly ($P<0.05$) higher than was the initial count. However, regardless of the packaging system under either abuse or control conditions, APCs at 7°C were lower on control livers than on abused livers.

Coagulase-positive *Staphylococcus aureus*, coliform, and *Clostridium perfringens* surface counts were all very low (<10 organisms/cm²) and no significant differences were observed between any of the treatments. *C. perfringens* was recovered from fresh beef tongues only. The absence of this microorganism following freezing probably resulted because *C. perfringens* is very susceptible to the low temperatures encountered in frozen storage (4). Generally, freezing is detrimental to *Enterobacteriacea*; however, occasionally only injury occurs, which can be repaired under thawing (abuse) conditions (1,16). Low coliform and *S. aureus* counts were probably a result of minimal contamination during evisceration. No *Enterobacteriacea* were recovered from the surfaces of beef tongues. KF Streptococcal surface counts were all very low and no significant ($P<0.05$) differences were observed between treatments. Since these organisms, in general, inhabit the intestinal contents of animals (3), one would not expect large numbers on the tongue itself.

Surface counts of anaerobic bacteria on beef tongues ranged from 2.59 to 4.46 organisms/cm² ($\log_{10}$). Naked product had significantly ($P<0.05$) higher counts than vacuum-packaged abused product and control treated samples. Surface counts on the control treatments were not significantly different from initial counts. The significant difference between vacuum-packaged abused product and the naked abused product could possibly be attributed to the presence of facultative anaerobes. Facultative anaerobes grow more rapidly under aerobic conditions, but will grow under anaerobic conditions as well (4). Thus it is likely that these organisms grew faster on the naked product, than on the vacuum-packaged product, once the tongues were subjected to temperature-abuse.

Yeast and mold counts were highest on naked and film-wrapped abused tongues. Initial yeast and mold counts were relatively low, and not significantly ($P<0.05$) different from counts obtained on tongues held under control conditions. Yeast and molds are slow growers and poor competitors, which might account for the low initial numbers but higher counts following storage and abuse conditions. The temperature used during these abuse conditions is more favorable to yeast and mold growth compared to bacterial growth (10).

Beef livers

Microbial analyses for beef livers after the simulated shipping and temperature-abuse are presented in Table 3. Highest APCs (35°C) were on naked, abused livers; however, there were not statistically significant differences in the APC at 35°C observed for any of the treatments. Aerobic plate counts at 20°C on fresh beef livers sampled initially averaged 3.58 organisms/cm² ($\log_{10}$), which was approximately one and one-half to two log cycles lower than counts previously reported for fresh beef livers (13,15). Variations in processing plant handling procedures could account for such discrepancies. As with beef tongues, results of microbial counts following the various treatments will change in accordance with the initial counts. Significantly ($P<0.05$) higher APCs at 20°C were observed on the surface of the film-wrapped and naked samples following abuse, compared to all other treatments. These results suggest that vacuum packaging significantly limits aerobic microbial growth if the product is subjected to abuse conditions similar to those of this study.

A significant ($P<0.01$) plant × packaging treatment interaction was found for aerobic plate counts at 7°C (Table 4). The APC at 7°C for plants I and II generally followed the same patterns between packaging, handling and storage conditions. However, bacterial counts for plant III tended to be lower than those of plants I and II. Furthermore, the vacuum-packaged control product from plant III showed approximately a one log cycle decrease in bacterial numbers from the initial count. The opposite result was found for vacuum-packaged product from plants I and II. The naked control samples from plant I increased more than one log cycle over the initial count, whereas for plants II and III, the counts for the naked control product were similar to the initial counts at these plants. Total bacterial counts at plant III were relatively low when compared to those of the other two plants. Beef livers obtained from plants I and II were washed with cold water post evisceration, while product obtained at plant III was subject to a warm (32°C) water rinse post evisceration. Most psychrotrophic bacteria are destroyed by a mild heat treatment (7). This could be a possible explanation as to why livers from plant III had lower APCs at 7°C (psychrotrrophic counts).
<table>
<thead>
<tr>
<th>Handling and storage conditions</th>
<th>35°C APC</th>
<th>20°C APC</th>
<th>Total Enterobacteriaceae</th>
<th>Staphylococcus aureus</th>
<th>Coliform</th>
<th>Clostridium perfringens</th>
<th>KF Streptococcal</th>
<th>Anaerobic</th>
<th>Yeast &amp; Mold</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INITIAL</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>3.69f</td>
<td>3.58g</td>
<td>0.30f</td>
<td>0.19g</td>
<td>0.95f</td>
<td>1.37f</td>
<td>4.00f</td>
<td>3.47f</td>
<td>0.51h</td>
<td></td>
</tr>
<tr>
<td><strong>ABUSE</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Vacuum packaging</td>
<td>4.14f</td>
<td>3.90g</td>
<td>0.41f</td>
<td>1.29f</td>
<td>0.63f</td>
<td>0.10g</td>
<td>2.52f</td>
<td>3.76f</td>
<td>1.14h</td>
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<td>Film wrapping, (PVC)</td>
<td>4.40f</td>
<td>4.54f</td>
<td>0.70f</td>
<td>1.64f</td>
<td>0.54f</td>
<td>0.06g</td>
<td>3.23f</td>
<td>4.25f</td>
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<tr>
<td>Naked (no wrapping material)</td>
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<td>4.93f</td>
<td>0.69f</td>
<td>1.79f</td>
<td>0.37f</td>
<td>0.00g</td>
<td>3.96f</td>
<td>4.31f</td>
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<td><strong>CONTROL</strong></td>
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<tr>
<td>Vacuum packaging</td>
<td>3.97f</td>
<td>3.64g</td>
<td>0.00f</td>
<td>1.34f</td>
<td>0.25f</td>
<td>0.35g</td>
<td>2.79f</td>
<td>3.42f</td>
<td>1.65gh</td>
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<td>3.57g</td>
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</table>

n = 27 livers for initial, 12 for abuse and 4 control conditions.

Counts = \( \log_{10}/\text{cm}^2 \).

APC = Aerobic plate count.

Two-four weeks storage at -29°C, followed by a simulated shipping-temperature abuse of 24 h at 22-28°C, followed by 13 days storage at -1 ± 0.05°C.

Two-four weeks storage at -29°C.

PVC = Polyvinyl chloride.

Means in the same column with different letters are significantly different at \( P<0.05 \).
TABLE 4. Aerobic plate counts at 7°C for beef livers according to the interaction of plant with packaging treatment.

<table>
<thead>
<tr>
<th>Packaging, handling, and storage conditions</th>
<th>Aerobic plate count at 7°C³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
</tr>
<tr>
<td>Plant I</td>
<td>2.38</td>
</tr>
<tr>
<td>Plant II</td>
<td>2.70</td>
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<tr>
<td>Plant III</td>
<td>1.60</td>
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<tr>
<td>Abused (vacuum packaged)⁴</td>
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<tr>
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<td>3.43</td>
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</tr>
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<td>4.04</td>
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<td>4.06</td>
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<tr>
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</tr>
<tr>
<td>Plant I</td>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<tr>
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<tr>
<td>Control (film wrapped)⁴</td>
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<tr>
<td>Control (naked)⁴</td>
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<tr>
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<td>Plant II</td>
<td>2.85</td>
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<tr>
<td>Plant III</td>
<td>1.45</td>
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³Count = log_{10}/cm²

⁴Two-four weeks storage at -29°C, followed by a simulated shipping-temperature abuse of 24 h at 22-28°C, followed by 13 days storage at -1 ± 0.5°C.

In conclusion, if frozen beef tongues and livers are subjected to a temperature-abuse similar to that of this study, vacuum packaging should allow for the least microbial proliferation.

**Acknowledgments**

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